7.—The Inheritance of Dorsal Pattern in Crinia Species (Anura Leptodactylidae)

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An analysis of the genetic factors controlling the inheritance of dorsal pattern in the polymorphic species *Crinia insignifera*, *C. glauerti*, *C. pseudinsignifera* and *C. georgiana* has been made by classifying the phenotypes of offspring from 75 crosses. The results are interpreted as indicating a simple genetic meehanism controlling the phenotype as follows: ridged, homozygous; warty and smooth, as for ridged but with modification of expression developing with age of young frogs; lyrate, heterozygous; weak lyrate, homozygous. The weak lyrate phenotype appears to be easily confused with the typical phenotype.

Introduction

Polymorphism in the genus *Crinia* has been discussed by Moore (1954, 1961) and Main (1961). I suggested (1961) that the morphs present on Rottnest Island could be interpreted as homozygous (phenotype ridged) and heterozygous (phenotype lyrate) for the genetic factors controlling dorsal pattern, and also pointed out that a complete analysis would depend on breeding animals for several generations. The results of these breeding experiments are given below. Numerous crosses were made with the idea of breeding from the F_1 animals; however, none has survived beyond the late summer of its first year. Despite the failure to complete that part of the experiment the results help in understanding the inheritance of the dorsal pattern. The length of larval life varies under laboratory conditions. *C. georgiana* with a larval life of upward of 40 days is the shortest while *C. insignifera* and *C. glauerti* are longest with 90-100 days. Larval mortality was proportional to the length of larval life, about 60% of eggs and larvae in *C. georgiana* metamorphosed. In *C. insignifera*, success fell to around 10%. The nett result of all these rearing experiments is that from 75 crosses about 1000 young frogs have been reared and categorised.

Description of Phenotypes

The phenotypes of *Crinia georgiana* (Tschudi) and *C. insignifera* Moore are illustrated in Figures 1 and 2. The phenotype in the other *Crinia* species are so similar to those of *C. insignifera* that illustration is not warranted.

At the commencement of my studies on the genus *Crinia* my primary interest lay in understanding the geographic and reproductive limits of each species. Consequently the analysis of the phenotypic variation within each species was left. However, I did keep records of the variation in each local population studied. In order to record the variation present I arbitrarily named certain phenotypes and defined their limits (Main, 1957: 31). The four categories were—ridged, smooth, lyrate, warty. In these phenotypic classes the dominant colour (which may be brown, fawn, gray, black) has been ignored and emphasis placed entirely on texture of the dorsal surface. This paper deals with the inheritance of phenotypic variation within each species.

As breeding and rearing progressed it became clear that the arbitrary categories did not coincide precisely with genetic characters. In particular, (a) the ridged and smooth phenotypes are connected by very infrequent intermediates; that these should be regarded as one category is supported by the experimental results, and (b) the warty phenotype has a genetic basis in *C. georgiana* different from that of other species, hence, the supposition that the phenotypes called "warty" are equivalent is unfounded. Experimentally, warty *C. georgiana* appears to be genetically similar to the ridged animals. In other species where the so-called "warty" phenotype occurs, it is not dark with rows of warts as in *C. georgiana*, but pale with irregular warts and indistinct light and dark patches dorsally. These pale, warty animals can be distinguished from smooth animals by the absence of dark flanks (Figure 1,2), and from lyrate animals by the absence of the crescentic ridges over the scapular region. In *Crinia insignifera*, so-called warty or weak lyrate animals, when crossed, produce offspring which suggest that qualitatively such animals are not lyrate in genotype. This last point will be discussed more fully in the results where all lyrate and weak lyrate parents have been listed in the same table.

Crosses

Matings were made as follows: (a) when a single female was to be mated to many males *in vitro* crosses were made by the method of Main (1957). In these cases the parents were preserved. (b) when a single male was mated to a single female the two animals were left clasping in a dish of water until the eggs were laid. Such crosses are called natural to distinguish from *in vitro* as used above. The parents were then removed, toe-clipped for individual identification and held outside in holding pens so that they could be used the following season for matings to their F_1s. Unfortunately these animals were not photographed as a record of their phenotype, consequently at present there is no way of confirming the phenotypic grading given when the mating was registered.

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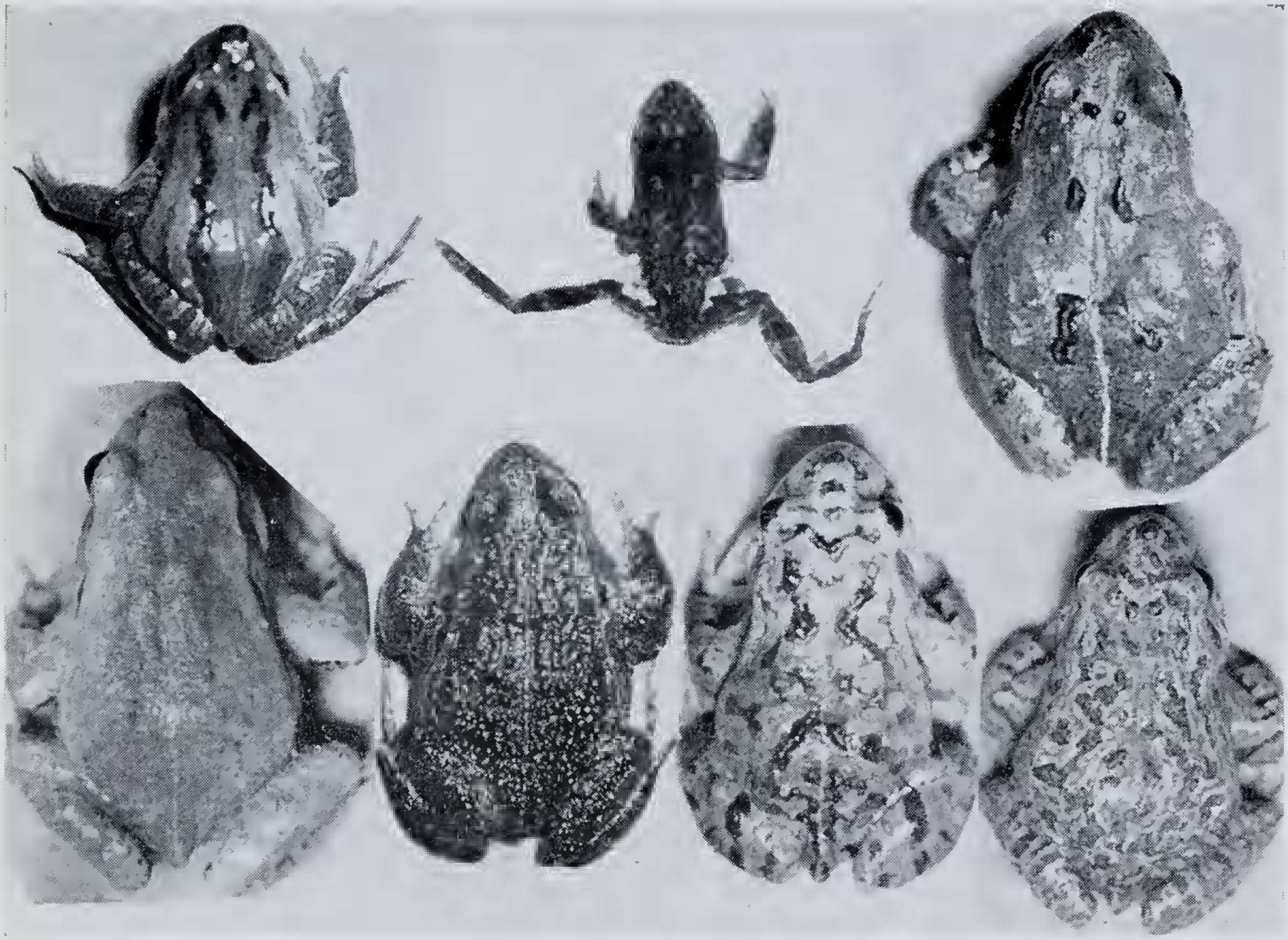

Figure 1.—Phenotypes of *Crinia georgiana* (Tschudi) Top row; (left to right) ridged, weak lyrate ♀ of cross 680 (Table 3,h), weak ridged. Bottom row; smooth, warty, lyrate.

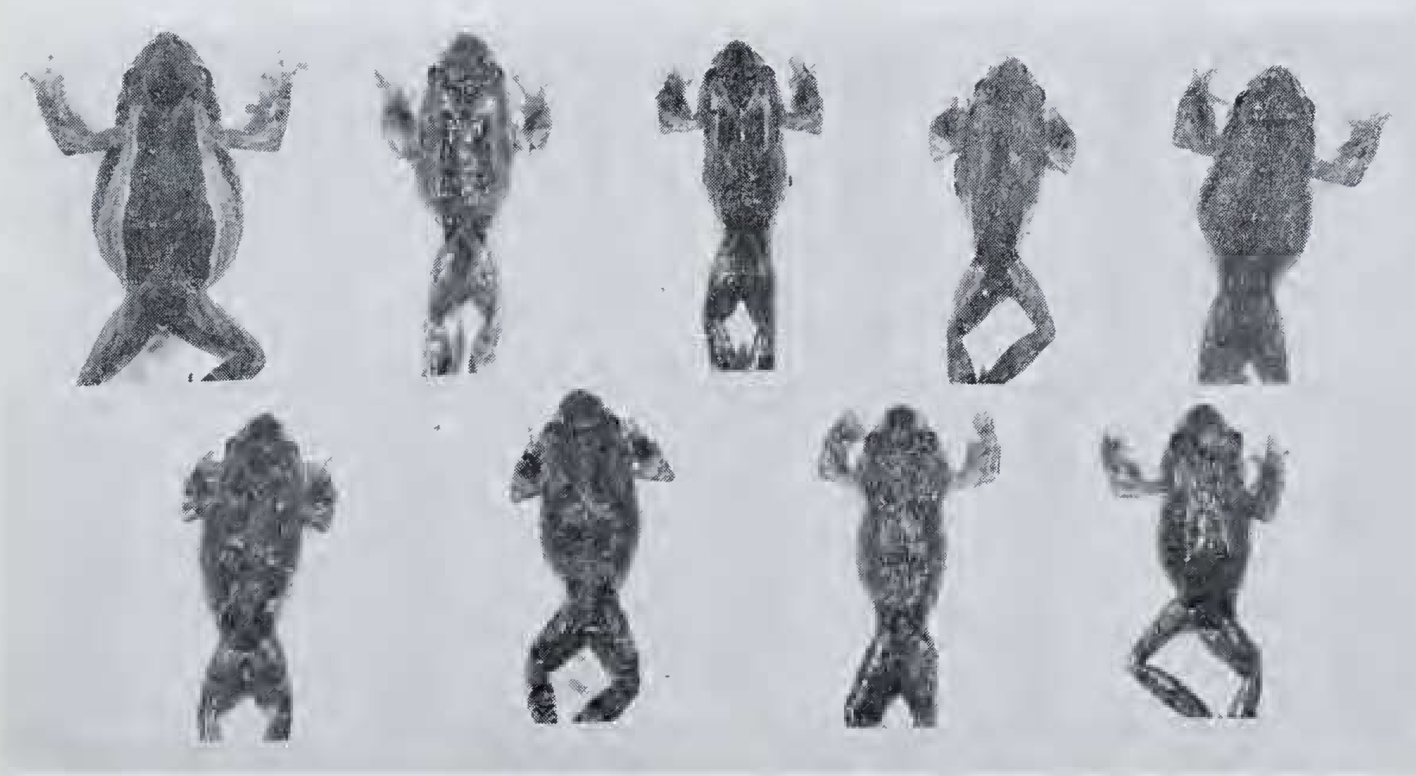

Figure 2.—Phenotypes of *Crinia insignifera*. Top row; (left to right) gradations from typical ridged to smooth. Bottom row; variations in expressions of lyrate phenotype.

TABLE 1

Phenotype of offspring resulting from crosses between various combinations of phenotypes of *Crinia insignifera.*

Cross No.	Parental combination	Offspring	
		Ridged	Lyrate
	a.		
462	Ridged ♀ x lyrate ♂	5	12
470		21	18
622		2	4
661		0	5
856		1	2
857		0	5
	Total . ..	29	46
	b.		
469	Lyrate ♀ x ridged ♂	0	6
475		7	5
716		2	0
733		12	11
810		0	1
902		0	1
	Total	21	24
	c.		
472	Lyrate ♀ x lyrate ♂	6	16
473		7	8
615		3	2
625		0	1
694		0	1
723		0	42
882		0	1
884		0	3
	Total	16	74

Results

Crinia insignifera

Table 1, a, gives results of ridged ♀ crossed to lyrate ♂. According to the hypothesis (Main 1961) the two phenotypes should be equally frequent. A test by chi square gives a value of 3.8 and p of almost 0.05 which is poor agreement with the hypothesis. The results of the reciprocal cross are given in Table 1, b. Again the two phenotypes were expected to be equally frequent among the offspring and the results are in good agreement with expectations.

The results presented in Tables 1, a and 1, b, reveal that from five crosses (661, 857, 469, 810 and 902) a total of 18 lyrate and no ridged offspring were produced. Several explanations can be offered to account for these results: (a) it may be simply chance, (b) the lyrate phenotype has a general superiority over the ridged; (c) if the genetic system controlling the dorsal pattern is at one locus, at which there may be one of two alleles, then in the crosses producing no ridged animals each parent may have been homozygous for a different allele. In this last case all the offspring would be heterozygous and lyrate in phenotype. Inspection of the original data reveals that the lyrate parent in each of the anomalous crosses was categorised as follows, 661 'normal', 857 'weak', 469 'weak', 810 'weak', 902 'normal'. 'Weak' lyrate parents do not appear to be associated with crosses from which ridged and lyrate offspring were obtained. This suggests that 'weak' lyrate animals are homozygous and not heterozygous and when the results of crosses 857, 469 and 810 are removed from the tables the results do not differ significantly from equality.

The results of crosses in which both parents were lyrate in phenotype are presented in Table 1, c. The phenotypes of offspring in cross 723 are unlikely to be the result of crossing two heterozygotes. At the time the phenotypes of the offspring were scored, 42 had metamorphosed and 26 were unmetamorphosed; the unmetamorphosed offspring could not be scored phenotypically. The metamorphosed frogs and late stage larvae were then kept for rearing. Ridged animals are not usually delayed in metamorphosis and there is not reason to believe that the late metamorphosing animals would differ from the first 42 in phenotype.

The female parent of cross No. 884 was categorised as 'weak' and parents used in the other crosses were categorised as 'normal'. If the results of crosses 723 and 884 are not considered because they could be interpreted as being between a heterozygote and homozygote from which no ridged animals could be obtained, all crosses in Table 1, c produced 16 ridged and 29 lyrate offspring. In the event that heterozygotes (lyrate) and homozygotes (weak lyrate) cannot be distinguished in freshly metamorphosed animals we would expect ridged and lyrate to be in a 1 : 3 ratio. Test by chi square gives p of slightly less than 0.05 indicating a poor fit and a deficiency of lyrate which may be due to chance or a deficiency of the homozygote (weak lyrate) phenotype.

There have been three crosses involving the smooth morph of *C. insignifera* in the present series of results. Firstly, in cross No. 738 where a ridged ♀ crossed to smooth ♂ produced 8 offspring categorised upon metamorphosis as ridged. However, as growth proceeded 4 of the ridged became indistinct and finally were categorised as smooth. The two other crosses involved the reciprocal cross of smooth and lyrate; No. 619 (smooth ♀ x lyrate ♂) produced 3 ridged and 2 lyrate offspring while No. 149 (lyrate ♀ x smooth ♂) yielded 2 ridged and 1 lyrate. The results of the last two crosses are qualitatively similar to crosses ridged x lyrate.

Crinia glauerti Loveridge

This species has a long larval life, and is consequently difficult to rear to metamorphosis. Only five crosses involving this species have produced offspring and the results are presented in Table 2. These results are consistent with those already presented for *C. insignifera*.

Crinia pseudinsignifera Main

This species does best under fluctuating environmental conditions and is difficult to rear under constant temperature conditions. There are results from only two kinds of combinations for this species: two crosses lyrate ♀ x ridged ♂ (Nos. 142 and 894) produced 3 offspring from each cross all being categorised lyrate; and one cross lyrate ♀ x lyrate ♂ (No. 888) produced 3 ridged and 4 lyrate offspring. These results are statistically consistent with those already presented for *C. insignifera*.

TABLE 2

Phenotype of offspring resulting from crosses between various combinations of phenotypes of *Crinia glauerti*.

Cross No.	Parental combination	Offspring Ridged	Lyrate	Smooth
	a.			
631	Ridged ♀ x ridged ♂	5	0	0
	b.			
626	Ridged ♀ x lyrate ♂	4	3	0
651		5	7	0
652		15	13	0
	Total	24	23	0
	c.			
629	Smooth ♀ x ridged ♂	9	0	8

Crinia georgiana

This species has a very short larval life and many crosses were made in the hope of rearing offspring to sexual maturity and so breeding from the F_1 animals.

Table 3a shows the phenotypes of offspring resulting from crossing ridged ♀ x ridged ♂, in all cases only ridged animals result. Table 3b gives the phenotype of ridged ♀ x lyrate ♂; in this case both categories were found in the offspring in equal proportions. There is only one reciprocal cross (No. 367); lyrate ♀ x ridged ♂ yielded 5 ridged and 2 lyrate offspring. No cross produced exclusively lyrate offspring so there are no difficulties of interpretation such as arose when discussing results in Table 1 for *C insignifera*.

The smooth and warty categories are more common in *C. georgiana* than in other Western Australian species of *Crinia*. For some time it was thought that the smooth animals may represent the recessive homozygote, in which case crosses of ridged x smooth should produce only lyrate offspring. Tables 3c and d show the results of such crosses; of 112 offspring none was lyrate, 12 were regarded as weakly ridged, the others normally ridged. This gives no support to the supposition that smooth animals represent the recessive homozygote.

Various combinations of other phenotypes crossed with smooth animals are shown in Table 3, c, f, g and h. The cross smooth x smooth produces only ridged or weakly ridged offspring and similar results arise in cross smooth x warty. At metamorphosis the offspring from the cross smooth x lyrate produce 2 phenotypes, ridged and lyrate. When these are reared some of the ridged animals turn into smooth. Ignoring the subsequent development of the offspring the results are not different from those obtained by crossing ridged x lyrate. However, one reciprocal cross, No. 680, (Table 3, h) produced 69 lyrate offspring from a total of 85 eggs. Such a result can only be interpreted if parents were each homozygous for the different alleles in the genetic system controlling dorsal pattern so that the F_1s were all heterozygous and lyrate in phenotype. Such a result suggests that the lyrate genotype is dominant in expression.

The results of crosses involving various combinations of ridged, warty and lyrate are shown in Table 3 i, j, k, l, m. Warty x lyrate crosses resemble ridged x lyrate crosses while ridged x warty resemble ridged x ridged.

TABLE 3

Phenotype of offspring resulting from crosses between various combinations of phenotypes of *C. georgiana*.

Cross No.	Parental combination	Offspring Ridged	Lyrate
	a.		
383	Ridged ♀ x ridged ♂	64	0
395		23	0
479		41	0
480		34	0
	Total	162	0
	b.		
129	Ridged ♀ x lyrate ♂	23	22
196		3	3
396		6	9
398		9	7
400		6	2
	Total	47	43
	c.		
370	Ridged ♀ x smooth ♂	4	0
662		33	0
768		28	0
	Total	65	0
	d.		
482	Smooth ♀ x ridged ♂	12	0
483		21	0
872		14	0
	Total	47	0
	e.		
202	Smooth ♀ x smooth ♂	4	0
222		12	0
848		16	0
	Total	32	0
	f.		
221	Smooth ♀ x warty ♂	12	0
484		17	0
	Total	29	0
	g.		
197	Smooth ♀ x lyrate ♂	4	1
205		1	8
224		8	2
391		6	4
402		7	3
408		5	7
497		3	11
	Total	34	36
	h.		
365	Lyrate ♀ x smooth ♂	1	3
680		0	69
847		0	1
	Total	1	73
	i.		
368	Lyrate ♀ x lyrate ♂	1	3
392		2	4
	Total	3	7

TABLE 3.—*continued.*

Cross No.	Parental combination	Offspring	
		Ridged	Lyrate
	j.		
390	Lyrate ♀ x warty ♂	3	4
394		14	7
	Total	17	11
	k.		
403	Warty ♀ x lyrate ♂	9	10
	Total	9	10
	l.		
405	Warty ♀ x ridged ♂	20	0
	Total	20	0
	m.		
481	Ridged ♀ x warty ♂	32	0
	Total	32	0

Discussion

The present results are fragmentary; there is not equal depth for all species nor even for all combinations of morphs. Nevertheless, it seems clear that in all species of *Crinia* so far tested the cross ridged x ridged produces only ridged offspring and ridged is then homozygous for the genetic factor producing this dorsal pattern. Results of crosses involving either lyrate x lyrate or lyrate x ridged suggest that lyrate can be interpreted as being heterozygous. Cross 680 (lyrate ♀ x smooth ♂, Table 3, h) is strong evidence contrary to this hypothesis and suggests that morphs categorised as lyrate include heterozygous and homozygous animals. Some results presented in Table 1 are in agreement with such a suggestion.

The problem of whether, in the field, the homozygote (weak lyrate) is lethal or occurs in very low frequency has not been resolved. Main (1961) believed it was lethal. This interpretation is not supported by the present data; it is more likely to be semi-lethal since the results of crosses 469, 723, 810 and 857, Table 1 and 680 Table 3, h suggest that a phenotype readily confused with lyrate is present in some populations. In crosses, such phenotypes produce results markedly different from crosses in which typical lyrate animals are used.

The present results have some bearing on the problem of the nature of the factors affecting the smooth and warty phenotype. In field observations and when categorising field caught animals, large series from a number of localities could be arranged in a way which suggested that no break between weakly ridged animals and patternless animals (Figures 1 and 2) occurred. The results already presented in Tables 2 and 3 suggest that warty and smooth are ridged animals in which there is an impairment of expression of the dorsal pattern. This interpretation is supported by the observation of individually marked young animals as they grew in the departmental yards. In a number of such observations the ridge pattern gradually became less and less distinct until finally the animals were categorised as warty or smooth. These results suggest that in scoring phenotype frequency in the field, all non-lyrate phenotypes can be grouped together.

The cause of the altered expression in the ridged genotype is not clear. It appears not to be related to temperature because changes in expression were observed in young frogs held at a constant temperature of 71°F., or 62°F., and in others held in the open in departmental yards. Frequency of occurrence of patternless animals in the field is variable; they are absent from Rottnest and their frequency tends to increase towards the south in *C. georgiana*. In *C. pseudinsignifera* they are relatively rare and decrease towards the east.

Acknowledgments

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